



# Garlic: An Alternative Treatment for Group B *Streptococcus*

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**ABSTRACT** Prenatal screening in pregnant women between 35 and 37 weeks of gestation and intrapartum antibiotic prophylaxis has successfully reduced the incidence of neonatal morbidity and mortality related to *Streptococcus agalactiae*. However, the contamination rates of newborns are still considerable. In traditional and folk medicines, it has been observed that garlic has been effective in treating *S. agalactiae* infection. The aim of this study was to isolate and identify the active compounds from garlic that have antimicrobial activity against *S. agalactiae*. In order to do this, SP80 (Sep-Pak 80%) obtained from crude garlic extract (CGE) was fractionated by reverse-phase ultrafast liquid chromatography with UV (RP-UFLC-UV) using a Shim-pack PREP-ODS column. All fractions obtained were tested using a microbial growth inhibition test against the *S. agalactiae* strain (ATCC 12386). Five clinical isolates were used to confirm the action of the fractions with antimicrobial activity, and the bacterial growth curve was determined. Identification of the antimicrobial compounds was carried out through liquid chromatography coupled with mass spectrometry (LC/MS) and nuclear magnetic resonance (NMR). The active compounds found to exhibit antimicrobial activity were  $\gamma$ -glutamyl-S-allyl-cysteine (fraction 18),  $\gamma$ -glutamyl-phenylalanine (fraction 20), and the two stereoisomers (*E* and *Z*) of ajoene (fraction 42). The MICs of these fractions were 5.41 mg/ml, 4.60 mg/ml, and 0.16 mg/ml, respectively, and they inhibited the growth of the clinical isolates tested. Antimicrobial compounds from garlic may be a promising source in the search for new drugs against *S. agalactiae*.

**IMPORTANCE** Invasive disease due to group B streptococcal (GBS) infection results in a wide spectrum of clinical disease in neonates. Maternal colonization by GBS is the primary risk factor for disease. The strategy recommended by the Centers for Disease Control to reduce neonatal GBS infection is the culture-based screening of all pregnant women at 35 to 37 weeks of gestation and intrapartum antibiotic prophylaxis (IAP). However, indiscriminate use of antibiotics favors the selection and spread of resistant bacteria. The global scenario of antibacterial resistance has been of great concern for public health, and natural products can be a source of new substances to help us grapple with this problem.

**KEYWORDS** *Allium sativum* L., *Streptococcus agalactiae*,  $\gamma$ -glutamyl-S-allyl-cysteine,  $\gamma$ -glutamyl-phenylalanine, ajoene, antimicrobial, antimicrobial activity, bacterial growth kinetics, organosulfur compounds

*Streptococcus agalactiae*, also known as group B *Streptococcus* (GBS) is a round Gram-positive coccus bacterium which inhabits the gastrointestinal and genitourinary tracts of humans as a commensal organism (1). In the 1970s, studies showed the

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pathogenicity of this microorganism, associating it with bacteremia, pneumonia, and meningitis in infants under 3 months of age (2).

Neonatal GBS disease is an invasive infection affecting newborns during the first weeks of life and is a major cause of morbidity and mortality during this period. It can be divided into two types. The first type is early-onset GBS disease (EOGBS), which manifests within the first 72 h of life and can be acquired through vertical transmission from mother to fetus before and during childbirth. Its symptoms include respiratory disease, sepsis, and meningitis. The second type is late-onset GBS disease (LOGBS), which emerges between the 4th and 90th days of life and is acquired through contact of the newborn with the environment, presenting with symptoms such as sepsis, meningitis, urinary tract infection, osteoarthritis, respiratory disease, and cellulitis (3).

In 1996, prophylactic recommendations by the American College of Obstetricians and Gynecologists (ACOG) (4) and the Centers for Disease Control and Prevention (CDC) (5) were implemented to prevent GBS infections in newborns, and in 1997, the American Academy of Pediatrics (AAP) issued its own guidelines (6). These included measures such as the use of antibiotics during childbirth.

In 2010, the CDC published a revised consensus opinion, recommending vaginal cultures for all pregnant women between 35 and 37 weeks of gestation, with intrapartum antibiotic prophylaxis (IAP) being given to at-risk women (7).

In the absence of IAP, around 50% of newborns of mothers with GBS positive cultures are colonized by *S. agalactiae*, and 1 to 2% can develop EOGBS disease (2). Although prophylactic measures are well established in conventional medicine, herbal-based medicines are widely used for disease prevention in developing countries such as Brazil, and there is a history in traditional medicine of the use of garlic for the treatment of GBS infection in pregnant women (8, 9).

Garlic (*Allium sativum* L.), part of the family Liliaceae, besides being widely used as a food and condiment, has a series of therapeutic properties attributed to it in traditional medicine (10), many of which have yet to be scientifically confirmed. This plant species has been described as having antioxidant (11), antitumoral (12), anti-inflammatory (13), immunomodulatory (14), antiviral (15), antimicrobial (16, 17), and cardiovascular protective (18) actions, as well as promoting beneficial effects in diabetic (19) and obese patients (20).

Organosulfur compounds are the main bioactive compounds present in garlic, with allicin, which is responsible for garlic's strong odor, being the most commonly described compound with medicinal activity in the literature (21).

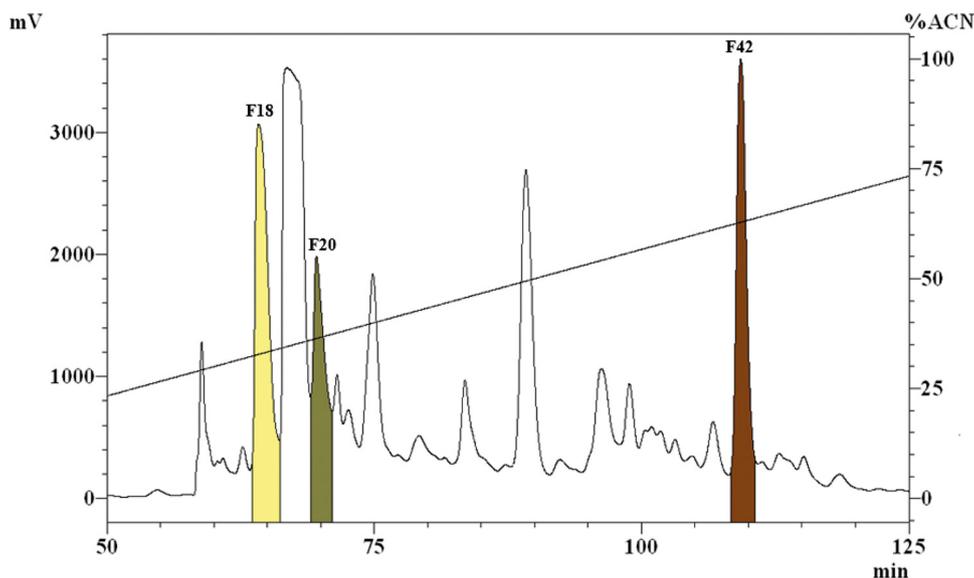
Allicin was identified by Cavallito and Bailey (22) and has been demonstrated to have antimicrobial activities against Gram-positive and Gram-negative bacteria (23). It is converted from its precursor alliin by the enzyme alliinase when tissue damage occurs. It is quite unstable and quickly participates in a cascade of nonenzymatic reactions to produce compounds such as vinylidithiins, ajoenes, and (poly)sulfides, which have been reported to exhibit antimicrobial activity (21, 24).

Ajoenes (*E* and *Z*) are also sulfur-containing compounds, and their antimicrobial activity has been demonstrated against Gram-positive and negative bacteria and fungi, such as *Aspergillus niger* and *Candida albicans* (24).

In view of the ethnopharmacological information and antimicrobial properties described to date, the objective of the present study was to assess the actions of garlic and its active principals isolated from the plant against *S. agalactiae* bacterium. The study sought to assess information gleaned from traditional medicine and isolate the chemical constituents with pharmacological activity, thereby expanding the armamentarium of treatment options available for the management of infections by the bacterium in pregnant women.

## RESULTS

**Isolation/purification.** The antimicrobial activity of *A. sativum* L. against *S. agalactiae* strain ATCC 12386 was investigated, revealing that incubation of crude garlic



**FIG 1** Chromatographic profile of SP80, obtained from Sep-Pak  $C_{18}$  cartridges. The analysis was performed using an RP-UFLC system (Shimadzu Prominence device) with a preparative reverse-phase column, Shim-pack PREP-ODS (250 mm  $\times$  50 mm inside diameter [i.d.], 15  $\mu$ m), and the absorbance was monitored at 225 nm. Note the peaks for fractions 18, 20, and 42 (F18, F20, and F42, respectively) that correspond to the fractions with antimicrobial activity against *S. agalactiae* ATCC 12386.

extract (CGE) on culture plates inhibited the bacterial growth. It was, therefore, then submitted to a solid-phase extraction (SPE) on Sep-Pak  $C_{18}$  cartridges, using 80% acetonitrile (ACN) + 0.05% trifluoroacetic acid (TFA) solution as the mobile phase. This step provided the SP80 fraction, which maintained antimicrobial activity against *S. agalactiae* ATCC 12386.

Reverse-phase ultrafast liquid chromatography with UV spectroscopy (RP-UFLC-UV) of the SP80 provided fractions according to retention time (Rt) and polarity. Biomonitoring of these fractions (46 in total) disclosed antimicrobial activity in only fractions 18, 20, and 42 (Fig. 1). The yields of these fractions were 8.48%, 2.77%, and 0.31%, respectively.

**Liquid growth inhibition assay and MIC.** CGE, SP80, and all fractions (25) were evaluated for antimicrobial activity against *S. agalactiae* ATCC 12386, and the microbial growth was measured by monitoring the increase in optical density at 595 nm ( $OD_{595}$ ) using a Victor3 1420 multilabel counter plate reader (Perkin Elmer, Waltham, MA, USA). The values are shown in Table 1. The MICs against *S. agalactiae* ATCC 12386 were determined for the fractions with antimicrobial activity (fractions 18, 20, and 42) and for CGE and SP80. These results are shown in Table 2 and are compared with the MIC of penicillin G as the positive control in the liquid growth inhibition assay. The results of these microbiological assays showed that the greatest growth inhibition was achieved when the bacteria of *S. agalactiae* ATCC 12386 were incubated with fraction 42.

Strains of clinical isolates of *S. agalactiae* provided by the Salomão Zoppi, Sao Paulo, SP, Brazil, were used in the evaluation of the antimicrobial activity of fractions 18, 20, and 42 against *S. agalactiae*. Using the MICs determined for *S. agalactiae* ATCC 12386, all fractions inhibited the growth of the clinical isolates tested.

**Bacterial growth curve kinetics.** Investigation of the bacterial growth kinetics in TSB medium was conducted to evaluate the antibacterial potency of fractions 18, 20, and 42 against *S. agalactiae* ATCC 12386. Figure 2 shows the optical density at 595 nm for the different incubation times. After 18 h of incubation, the negative-control (TSB) growth curve achieved exponential growth, while the fractions and penicillin G (positive control) showed no growth. The fractions and positive and negative controls were plated onto blood agar plates, and only the negative control showed bacterial growth.

**Mass spectrometry (LC-MS) and NMR.** After confirmation of the antimicrobial activity of fractions 18, 20, and 42, phytochemical analyses were performed to identify

**TABLE 1** Results of growth inhibition determined by measuring the absorbance at 595 nm using a Victor3 1420 instrument (Perkin Elmer) against *S. agalactiae* strain ATCC 12386 of all fractions obtained from bio-guided fractionation of SP80<sup>a</sup>

| Fraction                | OD           |
|-------------------------|--------------|
| F1                      | 0.239        |
| F2                      | 0.240        |
| F3                      | 0.225        |
| F4                      | 0.240        |
| F5                      | 0.211        |
| F6                      | 0.239        |
| F7                      | 0.234        |
| F8                      | 0.221        |
| F9                      | 0.211        |
| F10                     | 0.202        |
| F11                     | 0.262        |
| F12                     | 0.259        |
| F13                     | 0.258        |
| F14                     | 0.233        |
| F15                     | 0.250        |
| F16                     | 0.228        |
| F17                     | 0.213        |
| <b>F18</b>              | <b>0.036</b> |
| F19                     | 0.240        |
| <b>F20</b>              | <b>0.040</b> |
| F21                     | 0.247        |
| F22                     | 0.247        |
| F23                     | 0.228        |
| F24                     | 0.255        |
| F25                     | 0.229        |
| F26                     | 0.227        |
| F27                     | 0.224        |
| F28                     | 0.220        |
| F29                     | 0.219        |
| F30                     | 0.214        |
| F31                     | 0.241        |
| F32                     | 0.243        |
| F33                     | 0.227        |
| F34                     | 0.213        |
| F35                     | 0.218        |
| F36                     | 0.236        |
| F37                     | 0.242        |
| F38                     | 0.210        |
| F39                     | 0.220        |
| F40                     | 0.214        |
| F41                     | 0.243        |
| <b>F42</b>              | <b>0.038</b> |
| F43                     | 0.236        |
| F44                     | 0.216        |
| F45                     | 0.253        |
| F46                     | 0.221        |
| <b>CGE<sup>b</sup></b>  | <b>0.038</b> |
| <b>SP80<sup>c</sup></b> | <b>0.037</b> |

<sup>a</sup>Positive control (penicillin G): 0.036 optical density (OD; absorbance); negative control (sterile water and TSB), 0.215 OD. Fractions in bold are those that showed antimicrobial activity.

<sup>b</sup>CGE (crude garlic extract).

<sup>c</sup>SP80 were obtained after solid-phase extraction was performed using Sep-Pak C<sub>18</sub> cartridge, with 80% ACN + 0.05% TFA solution as mobile phase. F18, 20 and 42 fraction with antimicrobial activity.

the bioactive compounds present in these fractions. The data for fraction 18 were detected using liquid chromatography-diode array detection (LC-DAD) at 225 nm and a retention time (Rt) of 22.5 min in the total ion chromatogram (TIC) and liquid chromatography coupled with mass spectrometry (LC-MS) for a molecular ion peak of  $[M+H]^+ = 291.0981$  Da (Fig. 3a). The data for fraction 20 were detected using LC-DAD

**TABLE 2** Observed MIC for CGE, SP80, and antimicrobial fractions against *Streptococcus agalactiae* ATCC 12386

| <i>Allium sativum</i> L.  | MIC <sup>a</sup> (mg/ml) | SD   |
|---------------------------|--------------------------|------|
| CGE <sup>b</sup>          | 8.75                     | 0.04 |
| SP80 <sup>c</sup>         | 2.40                     | 0.42 |
| F18                       | 5.41                     | 0.79 |
| F20                       | 4.60                     | 0.69 |
| F42                       | 0.16                     | 0.02 |
| Penicillin G <sup>d</sup> | 0.038 $\mu$ g/ml         | 0.01 |

<sup>a</sup>MIC was performed in triplicate and expressed as mean  $\pm$  standard deviation.

<sup>b</sup>CGE, crude garlic extract.

<sup>c</sup>The SP80 sample was obtained after solid-phase extraction was performed using a Sep-Pak C<sub>18</sub> cartridge, with 80% ACN + 0.05% TFA solution as the mobile phase.

<sup>d</sup>Penicillin G, first-choice antibiotic for prophylaxis against *S. agalactiae* infection.

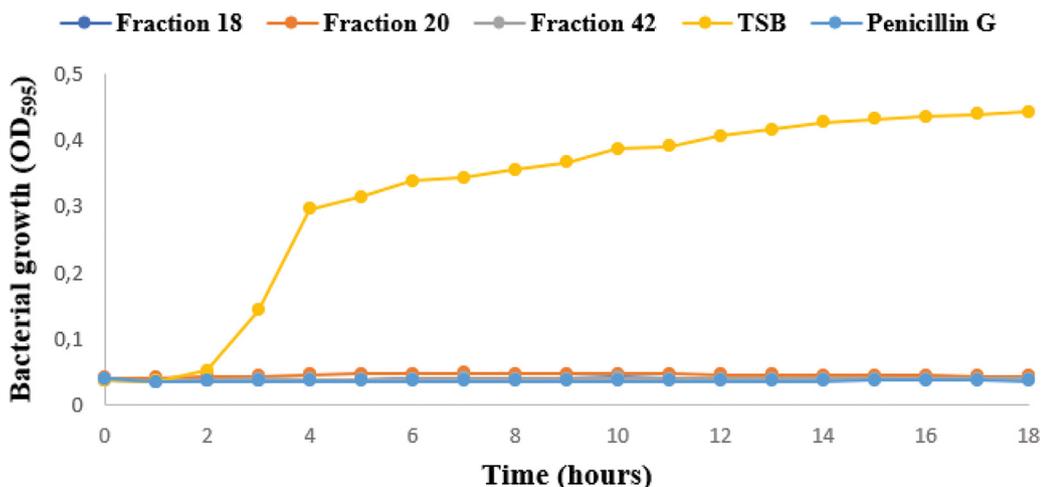
at 225 nm and an Rt of 25.8 min in the TIC and LC-MS for a molecular ion peak of  $[M+H]^+ = 295.1308$  Da (Fig. 3b). The data for fraction 42 were detected using LC-DAD at 225 nm and an Rt of 45.2 min in the TIC and LC-MS for molecular ion peaks of  $[M+H]^+ = 235.0311$  Da,  $[M+Na]^+ = 257.0131$ , and  $[2M+H]^+ = 469.0548$  Da (Fig. 3c).

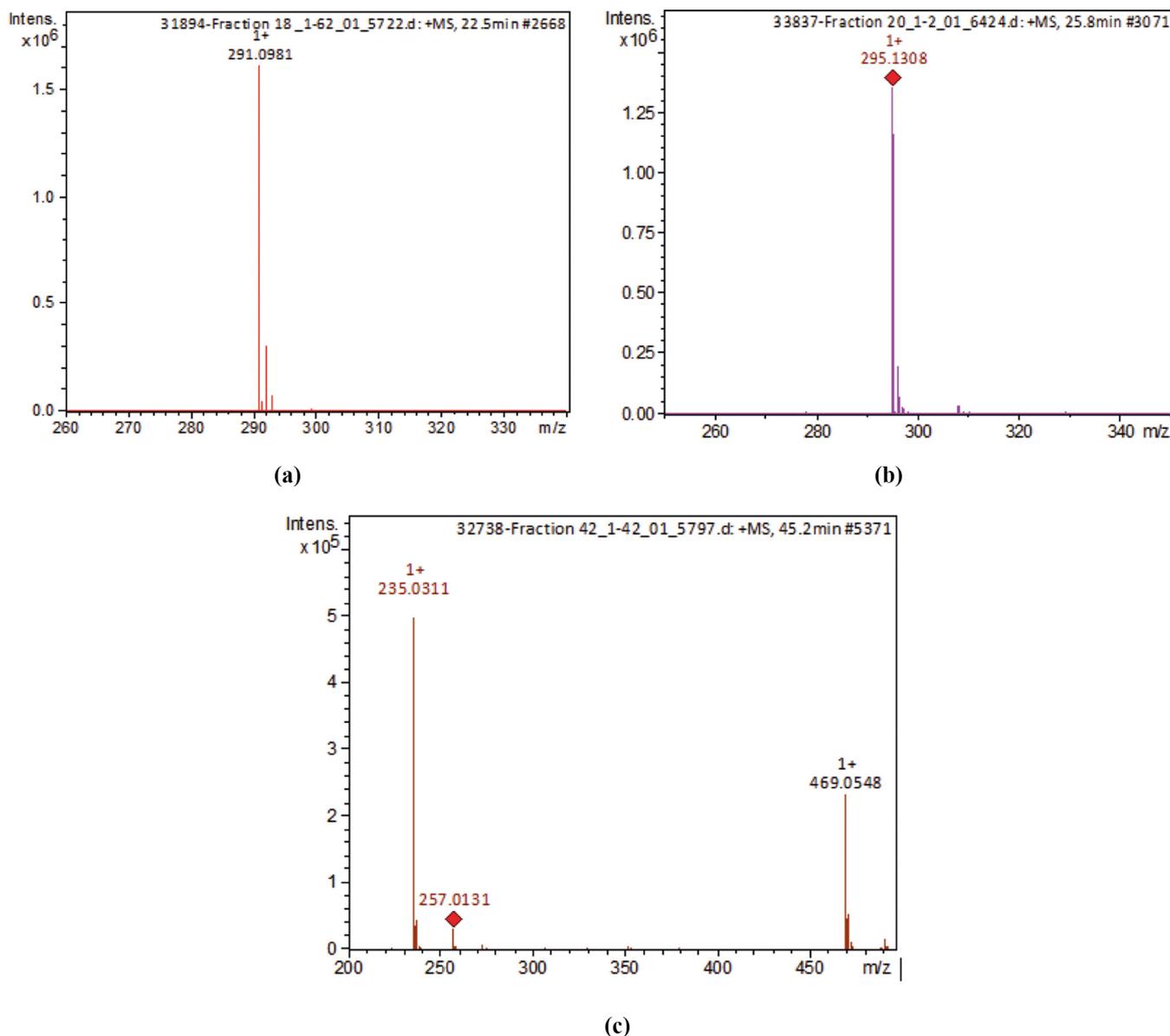
These data were confirmed by nuclear magnetic resonance (NMR). The results showed that fraction 18 corresponded to  $\gamma$ -glutamyl-S-allyl-cysteine (GSAC) (Fig. 4), fraction 20 to  $\gamma$ -glutamyl-phenylalanine (Fig. 5), and fraction 42 corresponded to the ajoenes E and Z (Fig. 6); these results were corroborated using the PubChem database (26, 27) (Tables 3, 4, and 5).

## DISCUSSION

The results of this study corroborate the ethnopharmacological information on the antimicrobial potential of *A. sativum* L. against group B *Streptococcus* (GBS). Although the antimicrobial actions of this plant species are well known, there is scant literature reporting its action against the pathogen (*S. agalactiae*) responsible for neonatal sepsis, which occurs due to vertical transmission from mother to fetus before or during childbirth (28, 29).

In addition to furnishing pharmacological evidence confirming the antimicrobial activity against *S. agalactiae* strains, this investigation allowed identification of the active principles present in *A. sativum* L. Although the compounds isolated in the study have previously been described in phytochemical studies, the relationship between these compounds and their actions against *S. agalactiae* are presented for the first time in this work (10, 30).

**FIG 2** Bacterial growth curve for *Streptococcus agalactiae* ATCC 12386 in the presence of fractions 18, 20, and 42, in tryptic soy broth (TSB) as the negative control.



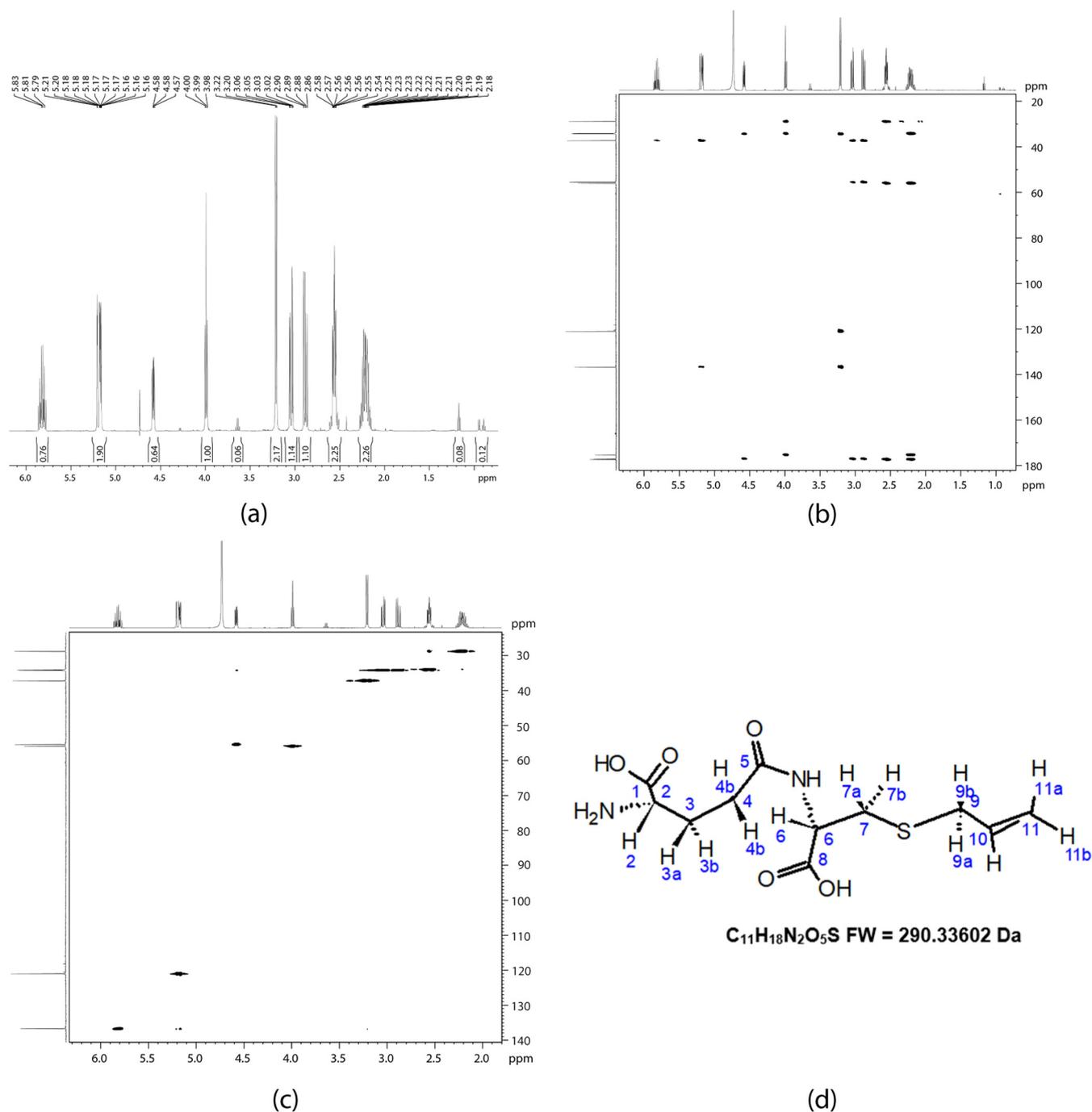
**FIG 3** Mass spectrometry (MS) using a Shimadzu model CBM-20A chromatograph. (a) Mass spectrum for  $\gamma$ -glutamyl-S-allyl-cysteine (GSAC) (fraction 18); (b) mass spectrum for  $\gamma$ -glutamyl-phenylalanine (fraction 20); and (c) mass spectrum for *E*- and *Z*-ajoenes (fraction 42).

Garlic contains two main biologically active substances: (i) organosulfur compounds like allicin, alliin, and ajoene and (ii) nonorganosulfur compounds (31). Allicin is the main organosulfur compound present in crushed garlic and exerts numerous biological actions, such as antimicrobial (32), anti-inflammatory (33), and antitumoral (34) effects.

The compounds with antimicrobial activity found in the present study are  $\gamma$ -glutamyl-S-allyl-cysteine (fraction 18),  $\gamma$ -glutamyl-phenylalanine (fraction 20), and *E*- and *Z*-ajoenes (fraction 42). Identification of these compounds was made possible using mass spectrometry (MS) and nuclear magnetic resonance (NMR) for the conformational and structural elucidation of the small molecules found in our study.

$\gamma$ -glutamyl-S-allyl-cysteine (fraction 18) is an organosulfur peptide present predominantly in whole garlic and undergoes hydrolysis and oxidation via *S*-allyl-L-cysteine (SAC) to form alliin. When garlic is crushed, alliin is transformed into allicin through the action of the alliinase enzyme (21, 35).

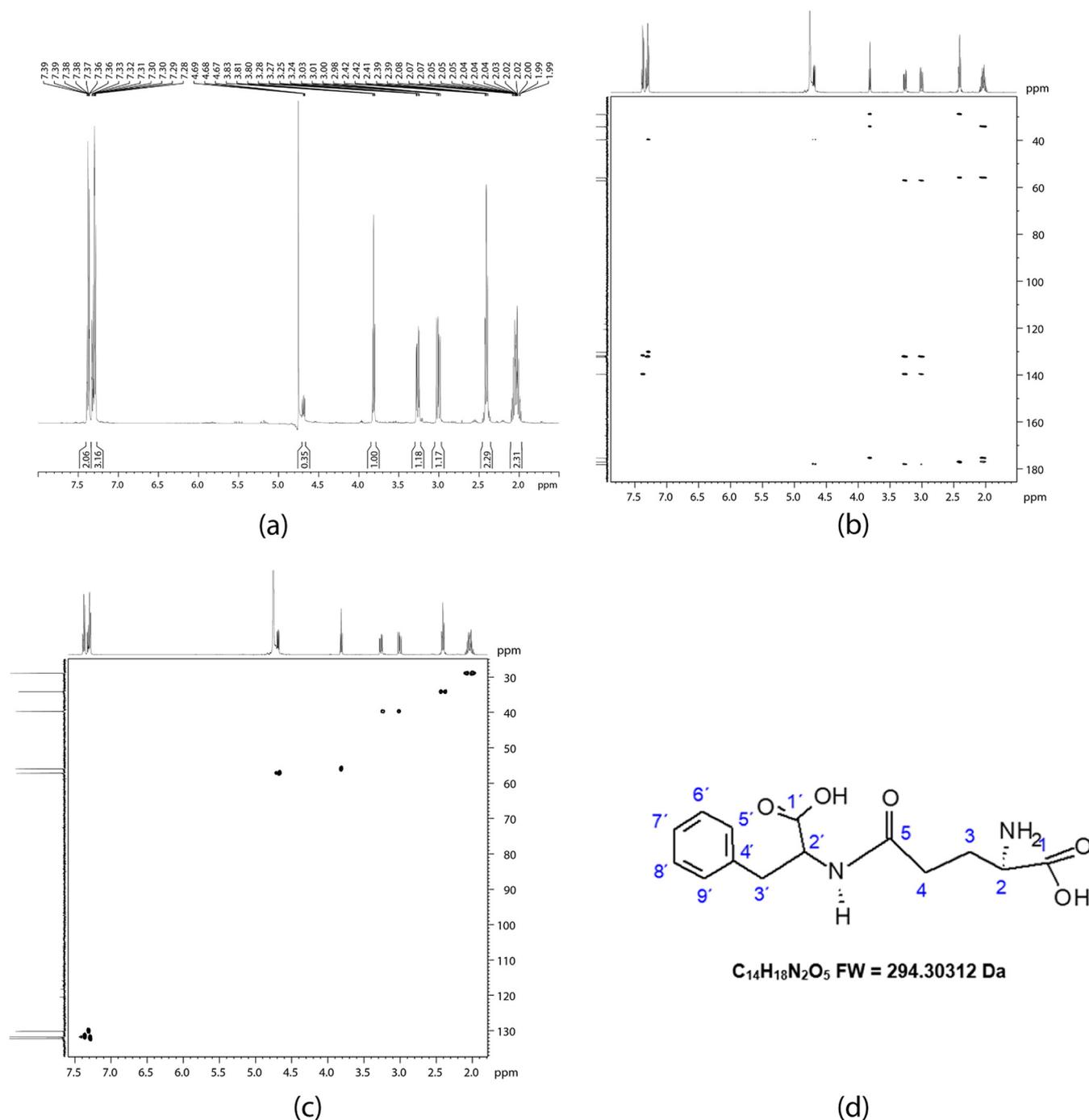
Amagase (36) concluded that  $\gamma$ -glutamyl-S-allyl-cysteine is converted into *S*-allyl-L-cysteine (SAC) through an enzyme reaction with  $\gamma$ -glutamyl-transpeptidase when garlic



**FIG 4** Nuclear magnetic resonance (NMR) spectra using a Bruker Daltonics maXis 3G instrument (500 MHz) in deuterated water ( $\text{D}_2\text{O}$ ): (a) 1D  $^1\text{H}$  NMR spectrum showing the multiplicity and the coupling constants; (b) 2D HMBC spectrum, providing correlations between carbons that are mainly separated by two or three bonds; (c) 2D HSQC spectrum—several dots are present, with each one representing a correlation between  $^1\text{H}$  and  $^{13}\text{C}$ ; and (d) chemical structure for  $\gamma$ -glutamyl-S-allyl-cysteine (fraction 18). FW, formula weight.

is extracted in an aqueous solution. Yeh et al. (37) also isolated and identified several organosulfur compounds, including  $\gamma$ -glutamyl-S-allyl-cysteine (GSAC),  $\gamma$ -glutamyl-S-methylcysteine (GSMC), and  $\gamma$ -glutamyl-S-propylcysteine (GSPC).

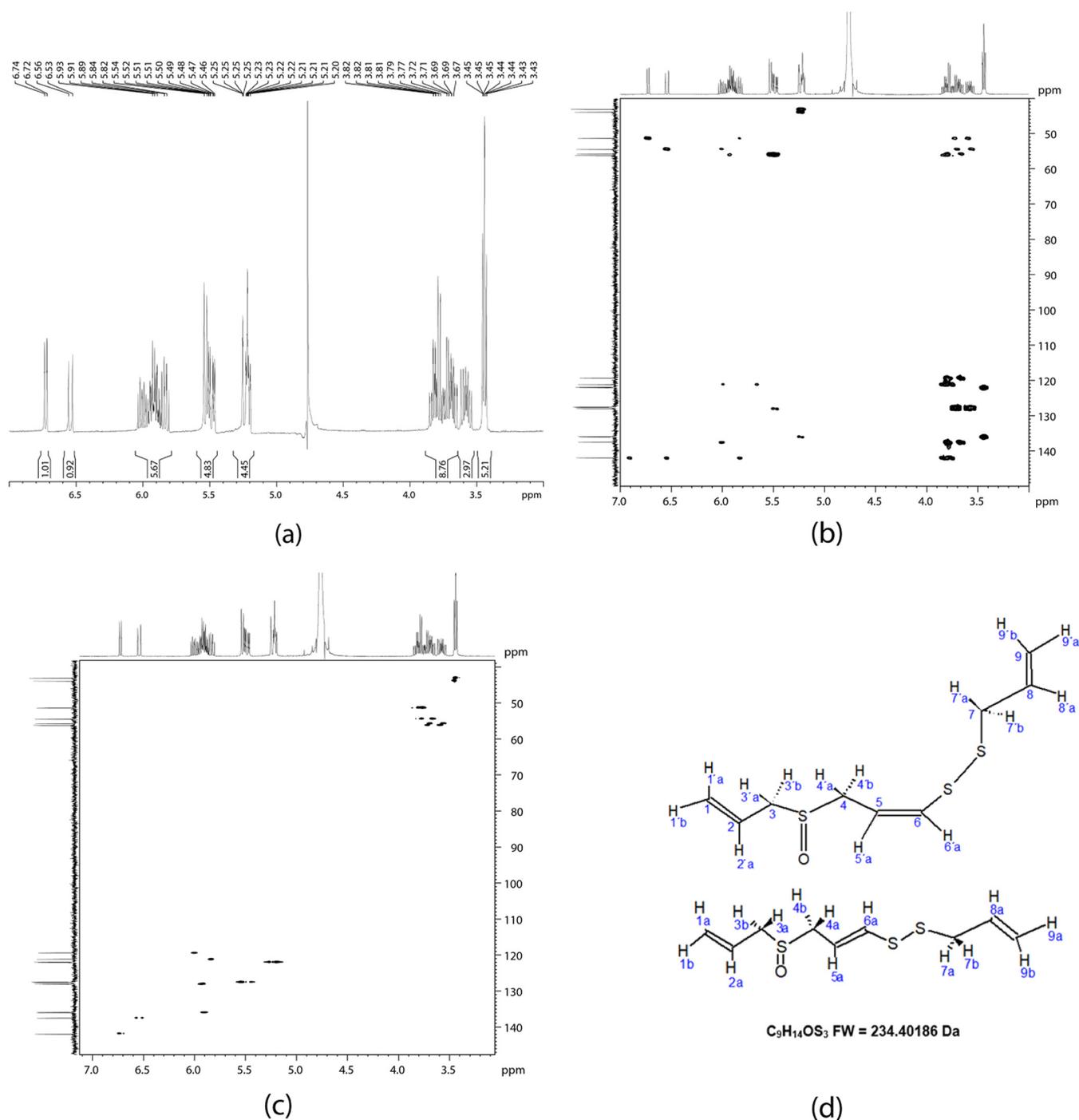
A study conducted by Zhu et al. (30) identified four organosulfur compounds present in garlic using the mass spectrometry method. One of these compounds was  $\gamma$ -glutamyl-S-allyl-cysteine, which was also identified in the present study as fraction 18. The mass spectrum obtained for this compound by the cited authors is the same spectrum obtained in the present study.



**FIG 5** Nuclear magnetic resonance (NMR) spectra (Bruker Daltonics maXis 3G instrument, 500 MHz) in deuterated water ( $\text{D}_2\text{O}$ ): (a) 1D  $^1\text{H}$  NMR spectrum showing the multiplicity and the coupling constants; (b) 2D HMBC spectrum, providing correlations between carbons that are mainly separated by two or three bonds; (c) 2D HSQC spectrum—several dots are present, with each one representing a correlation between  $^1\text{H}$  and  $^{13}\text{C}$ ; and (d) chemical structure for  $\gamma$ -glutamyl-phenylalanine (fraction 20). FW, formula weight.

There is a dearth of knowledge on the therapeutic effects of  $\gamma$ -glutamyl-*S*-allyl-cysteine, but it is known to promote alliin stores in whole garlic and can also be found in processed garlic (35).

Fraction 20 was identified as  $\gamma$ -glutamyl-phenylalanine, a peptide previously cited in the studies of Yoo et al. (10). However, in a review of the literature, no other studies involving this compound or reporting its biological actions were identified. Therefore, the current study is the first to report the antimicrobial action of this compound against *S. agalactiae*.



**FIG 6** Nuclear magnetic resonance (NMR) spectra (Bruker Daltonics maXis 3G instrument, 500 MHz) in deuterated water ( $\text{D}_2\text{O}$ ): (a)  $1\text{D } ^1\text{H}$  NMR spectrum showing the multiplicity and the coupling constants; (b) 2D HMBC spectrum, providing correlations between carbons that are mainly separated by two or three bonds; (c) 2D HSQC spectrum—several dots are present, with each one representing a correlation between  $^1\text{H}$  and  $^{13}\text{C}$ ; and (d) chemical structure for Z-ajoene (top) and E-ajoene (bottom) (fraction 42). FW, formula weight.

In the present study, fraction 42 proved the most effective fraction for antimicrobial action against *S. agalactiae*, compared to fractions 18 and 20. This fraction was identified as an ajoene (E and Z) mixture, comprised of organosulfur compounds that are formed by the degradation of pure allicin. This fraction is more stable than allicin. There are numerous studies on ajoene, including those describing actions such as antitumoral (34), apoptotic (38), antifungal (39, 40), antibacterial (24, 41), and antiplatelet

**TABLE 3** Uni- and bidimensional NMR in deuterated water (D<sub>2</sub>O) for fraction 18 ( $\gamma$ -glutamyl-S-allyl-cysteine), isolated from garlic<sup>a</sup>

| Carbon no. | Functional group         | <sup>1</sup> H ( $\delta$ ) or ppm                            | m, J = Hz <sup>b</sup>                                                                                                                        | HSQC ( <sup>13</sup> C) $\delta$ | HMBC $\delta$ <sup>1</sup> H $\delta$ (J <sup>3</sup> , J <sup>2</sup> )                                                                                                                                                                                        |
|------------|--------------------------|---------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1          | C=O                      |                                                               |                                                                                                                                               | 175.3                            | 2.2 <sup>1</sup> H <sub>3a,b</sub> (J <sup>3</sup> )<br>2.6 <sup>1</sup> H <sub>4a,b</sub> (J <sup>3</sup> )<br>3.9 <sup>1</sup> H <sub>2</sub> (J <sup>2</sup> )                                                                                               |
| 2          | CH                       | 3.9 H <sub>2</sub>                                            | t, J = 6.45 Hz                                                                                                                                | 55.8                             | 2.2 <sup>1</sup> H <sub>3a,b</sub> (J <sup>2</sup> )<br>2.6 <sup>1</sup> H <sub>4a,b</sub> (J <sup>3</sup> )                                                                                                                                                    |
| 3          | CH <sub>2</sub>          | 2.2 H <sub>3a</sub> , H <sub>3b</sub>                         | m (dq)                                                                                                                                        | 28.9                             | 2.6 <sup>1</sup> H <sub>4a,b</sub> (J <sup>2</sup> )<br>3.9 <sup>1</sup> H <sub>2</sub> (J <sup>2</sup> )                                                                                                                                                       |
| 4          | CH <sub>2</sub>          | 2.6 H <sub>4a</sub> , H <sub>4b</sub>                         | m (dt)                                                                                                                                        | 34.0                             | 2.2 <sup>1</sup> H <sub>3a,b</sub> (J <sup>2</sup> )<br>3.9 <sup>1</sup> H <sub>2</sub> (J <sup>3</sup> )                                                                                                                                                       |
| 5          | C=O                      |                                                               |                                                                                                                                               | 170.0                            | 2.2 <sup>1</sup> H <sub>3a,b</sub> (J <sup>3</sup> )<br>4.6 <sup>1</sup> H <sub>6</sub> (J <sup>3</sup> )                                                                                                                                                       |
| 6          | C <sub>6</sub> H (NH)-CH | 4.6 H <sub>6</sub>                                            | dd J = 4.8 and 4.6 Hz                                                                                                                         | 55.4                             | 3.05 <sup>1</sup> H <sub>7a,7b</sub> (J <sup>2</sup> )<br>3.29 <sup>1</sup> H <sub>9a,b</sub> (J <sup>3</sup> )                                                                                                                                                 |
| 7          | CH <sub>2</sub>          | 2.87 H <sub>7a</sub><br>3.05 H <sub>7b</sub>                  | dd J = 3.05 H <sub>7a</sub> J = 4.8 Hz and<br>4.8 Hz and J = 13.5 Hz<br><sup>1</sup> H-C <sub>7a</sub> = 2.87 ppm. dd J = 8.20<br>and 13.5 Hz | 34.2                             | 3.29 <sup>1</sup> H <sub>9a,b</sub> (J <sup>3</sup> )<br>4.6 <sup>1</sup> H <sub>6</sub> (J <sup>2</sup> )                                                                                                                                                      |
| 8          | C=O                      |                                                               |                                                                                                                                               | 177.1                            | 2.87 <sup>1</sup> H <sub>7a</sub> (J <sup>3</sup> )<br>3.05 <sup>1</sup> H <sub>7b</sub> (J <sup>3</sup> )                                                                                                                                                      |
| 9          | CH <sub>2</sub> -S       | 3.29 (H <sub>9a</sub> and<br>H <sub>9b</sub> )                | d, J = 7.20 Hz                                                                                                                                | 37.2                             | 2.87 <sup>1</sup> H <sub>7a</sub> (J <sup>3</sup> ); 3.05 <sup>1</sup> H <sub>7b</sub> (J <sup>3</sup> ); 5.20 (J <sup>3</sup> )<br><sup>1</sup> H <sub>11a (trans)}</sub> , 5.17 <sup>1</sup> H <sub>11b (cis)}</sub> and 5.83<br><sup>1</sup> H <sub>10</sub> |
| 10         | (CH=)                    | 5.83 H <sub>10</sub>                                          | dq                                                                                                                                            | 136.8                            | 3.29 <sup>1</sup> H <sub>9a,b</sub> ; 5.20 <sup>1</sup> H <sub>11a (trans)}</sub><br>5.17 <sup>1</sup> H <sub>11b (cis)}</sub>                                                                                                                                  |
| 11         |                          | 5.20 H <sub>11a (trans)</sub><br>5.17 H <sub>11b (cis)}</sub> | C <sub>11</sub> -H <sub>11b (geminal)</sub> and H <sub>11a (transvicinal)</sub>                                                               | 121.0                            | 3.29 <sup>1</sup> H <sub>9a,b</sub> (J <sup>3</sup> )                                                                                                                                                                                                           |

<sup>a</sup>NMR performed using a Bruker Daltonics maXis 3G instrument, 500 MHz.<sup>b</sup>m, multiplet; dq, doublet of quartets; dt, doublet of triplets; dd, doublet of doublets; d, doublet.

(42) activity. The activity-structure relationships of ajoene have also been established for antitumoral, antifungal, and antithrombotic actions (43), but no studies reporting the antibacterial activity of the ajoene mixture (fraction 42) against *S. agalactiae* bacterium were found.

Using the MIC value determined for *S. agalactiae* ATCC 12386, the fractions with antimicrobial activity inhibited the growth of all the clinical isolates tested and the bacterial growth curve using 3 $\times$  the MIC, confirming the antimicrobial activity of these fractions.

Cutler et al. (28) presented a study about the antimicrobial activity of allicin, a compound also found in processed garlic. This study differs from ours in relation to the number of strains used to determine the MIC and the compound used against the *S. agalactiae* strains. Comparing the MIC of the allicin (values ranging from 35 to 95 mg/liter) with the MIC of the fractions identified in our study (Table 1), the allicin was more efficient against the strains of *S. agalactiae*. However, our findings are also important, as we have presented new compounds with antibacterial activity against this *S. agalactiae* strain that have not previously been described in the literature and are more stable than allicin (34).

Hossain et al. (44) conducted a study to evaluate the antifungal activity of five isolated compounds from *Eremophila alternifolia* against eight yeast and two mold species. This study is like ours in relation to the method used to determine the MIC of the compounds (in triplicate); for this reason, it was used as a preliminary screening technique for antimicrobial activity.

Penicillin G is the first antibiotic of choice for intrapartum prophylaxis in pregnant women, followed by first-generation ampicillin and cephalosporin. In cases of patients allergic to penicillin G, options include clindamycin, erythromycin, and vancomycin (45). Velázquez et al. (46) analyzed 96 strains of *S. agalactiae*, all of which were sensitive to penicillin G at an MIC ranging from 0.012 to 0.094  $\mu$ g/ml, results corroborated by the current study findings. Kaminska et al. (25) analyzed 165 strains of clinical isolates

**TABLE 4** Uni- and bidimensional NMR in deuterated water (D<sub>2</sub>O) for fraction 20 (γ-glutamyl-phenylalanine), isolated from garlic<sup>a</sup>

| Carbon no. | Functional group       | <sup>1</sup> H (δ) or ppm                       | m, J = Hz <sup>b</sup>                | HSQC ( <sup>13</sup> C) δ | HMBC δ <sup>1</sup> H δ (J <sup>β</sup> , J <sup>γ</sup> )                                                                                      |
|------------|------------------------|-------------------------------------------------|---------------------------------------|---------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| 1          | C=O                    |                                                 |                                       | 175.5                     | 2.06 <sup>1</sup> H <sub>3a,b</sub> (J <sup>β</sup> )<br>3.80 <sup>1</sup> H <sub>2b</sub> (J <sup>γ</sup> )                                    |
| 2          | CH                     | 3.80 H <sub>2</sub>                             | 3.82 d, J = 10 Hz<br>3.80 d, J = 5 Hz | 56.0                      | 2.06 <sup>1</sup> H <sub>3a,b</sub> (J <sup>β</sup> )<br>2.48 <sup>1</sup> H <sub>4a,b</sub> (J <sup>β</sup> )                                  |
| 3          | CH <sub>2</sub>        | 2.06 H <sub>3a,b</sub>                          | dq 10 Hz                              | 29.0                      | 3.80 <sup>1</sup> H <sub>2b</sub> (J <sup>γ</sup> )<br>2.48 <sup>1</sup> H <sub>4a,b</sub> (J <sup>β</sup> )                                    |
| 4          | CH <sub>2</sub>        | 2.48 H <sub>4a,b</sub>                          | dq                                    | 34.0                      | 3.80 <sup>1</sup> H <sub>2b</sub> (J <sup>γ</sup> )<br>2.06 <sup>1</sup> H <sub>3a,b</sub> (J <sup>β</sup> )                                    |
| 5          | C=O                    |                                                 |                                       | 177.0                     | 2.02 <sup>1</sup> H <sub>3a,b</sub> (J <sup>β</sup> )<br>2.41 <sup>1</sup> H <sub>4a,b</sub> (J <sup>β</sup> ) (J <sup>γ</sup> )                |
| 1'         | C=O                    |                                                 |                                       | 179.0                     | 2.02 <sup>1</sup> H <sub>3'a</sub> <sup>1</sup> H <sub>3'b</sub><br>4.68 <sup>1</sup> H <sub>2'b</sub> dd (J <sup>γ</sup> )                     |
| 2'         | CH                     | 4.68 H <sub>2'b</sub>                           |                                       | 57.0                      | 3.00 <sup>1</sup> H <sub>3'a</sub> (J <sup>β</sup> )<br>3.25 <sup>1</sup> H <sub>3'b</sub> (J <sup>β</sup> )                                    |
| 3'         | CH <sub>2</sub>        | 3.00 H <sub>3'a</sub> and 3.25 H <sub>3'b</sub> | dd, J = 5 Hz                          | 40.0                      | 4.68 <sup>1</sup> H <sub>2'b</sub> dd (J <sup>γ</sup> )<br>7.28 H <sub>5'</sub>                                                                 |
| 4'         | CH <sub>aromatic</sub> |                                                 |                                       | 140.0                     | 3.00 <sup>1</sup> H <sub>3'a</sub> 3.25 <sup>1</sup> H <sub>3'b</sub> (J <sup>β</sup> )<br>4.68 <sup>1</sup> H <sub>2'b</sub> (J <sup>β</sup> ) |
| 5' and 9'  | CH <sub>aromatic</sub> | 7.28 H <sub>5'</sub> and H <sub>9'</sub>        | m                                     | 132.0                     | 3.00 <sup>1</sup> H <sub>3'a</sub> (J <sup>β</sup> )<br>3.25 <sup>1</sup> H <sub>3'b</sub> (J <sup>β</sup> )                                    |
| 6' and 8'  | CH <sub>aromatic</sub> | 7.36                                            | m                                     | 131.9                     | 3.00 <sup>1</sup> H <sub>3'a</sub> (J <sup>β</sup> )<br>3.25 <sup>1</sup> H <sub>3'b</sub> (J <sup>β</sup> )                                    |
| 7'         | CH <sub>aromatic</sub> | 7.30 H <sub>5'</sub> and H <sub>9'</sub>        | m                                     | 130.0                     | 7.28 <sup>1</sup> H <sub>5'</sub> and H <sub>9'</sub> (J <sup>β</sup> )                                                                         |

<sup>a</sup>NMR performed using a Bruker Daltonics maXis 3G instrument, 500 MHz.<sup>b</sup>m, multiplet; dq, doublet of quartets; dt, doublet of triplets; dd, doublet of doublets; d, doublet.

of *S. agalactiae* and all were sensitive to penicillin G, with an MIC ranging from 0.032 to 0.125 μg/ml. These studies are corroborated by the findings of the present work, which observed an MIC of 0.038 μg/ml.

The present study results showed that the antimicrobial effect of penicillin G was superior to that of the fractions identified. However, we believe it is important to expand the armamentarium of treatment options against GBS, particularly in cases of pregnant women allergic to penicillin G and given that IAP is effectively a prophylactic as opposed to a treatment. Moreover, the global scenario of antibacterial resistance has been of great concern for public health, and natural products can be a source of new substances to help us grapple with this problem. However, further preclinical trials assessing the pharmacodynamic, pharmacokinetic, and toxicological aspects of these substances are required to provide a basis for full clinical trials. These research efforts can pave the way for use of *A. sativum* L. or its isolated active principles, for example in a topical cream for vaginal use as a monotherapy or in association with antibiotics as a complementary therapy.

New compounds isolated from garlic with antimicrobial activity, in addition to alliin, were also discovered. This work will continue with respect to these molecules (commercially) to expand the activity spectrum, as well as for testing with a large number of clinical isolates of *S. agalactiae*.

## CONCLUSIONS

This study found that two peptides and an ajoene (E and Z) mixture were the most efficient compounds isolated from *A. sativum* L. (garlic) with respect to antimicrobial activity against *S. agalactiae*. These are extremely important findings, as they contribute to the possible development of a new medication for the topical treatment of *S. agalactiae* infection during pregnancy, instead of using prophylactic prevention methods during childbirth. The future development of such a treatment could help to avoid the indiscriminate use of antibiotics and the associated growth in bacterial resistance.

## MATERIALS AND METHODS

**Plant material.** The study was carried out in the Department of Physiological Sciences, Santa Casa de São Paulo School of Medical Sciences, São Paulo, SP, Brazil, and the Laboratory for Applied Toxinology at the Butantan Institute, São Paulo, SP, Brazil.

**TABLE 5** Uni- and bidimensional NMR in deuterated water (D<sub>2</sub>O) for fraction 42 (*E*- and *Z*-ajoenes), isolated from garlic<sup>a</sup>

| Data for <i>E</i> -ajoene: |                          |                                               |                                                              |                           |                                                                                                       |            |                          |                                               |                                                          |                           |                                                                                                          |
|----------------------------|--------------------------|-----------------------------------------------|--------------------------------------------------------------|---------------------------|-------------------------------------------------------------------------------------------------------|------------|--------------------------|-----------------------------------------------|----------------------------------------------------------|---------------------------|----------------------------------------------------------------------------------------------------------|
| Carbon no.                 | Functional group         | <sup>1</sup> H (δ) or ppm                     | m, <i>J</i> = Hz <sup>b</sup>                                | HSQC ( <sup>13</sup> C) δ | HMBC <sup>1</sup> H δ ( <i>J</i> <sup>3</sup> , <i>J</i> <sup>2</sup> )                               | Carbon no. | Functional group         | <sup>1</sup> H (δ) or ppm                     | m, <i>J</i> = Hz <sup>b</sup>                            | HSQC ( <sup>13</sup> C) δ | HMBC <sup>1</sup> H δ ( <i>J</i> <sup>3</sup> , <i>J</i> <sup>2</sup> )                                  |
| 1                          | CH <sub>2</sub> =<br>CH= | 5.53 H <sub>1a</sub> and 5.48 H <sub>1b</sub> | d, <i>J</i> = 10 Hz<br>dq, <i>J</i> = 15 Hz, 10 Hz, and 5 Hz | 127.0                     | 3.60, 3.73 H <sub>3a,b</sub> ( <i>J</i> <sup>3</sup> )                                                | 1'         | CH <sub>2</sub> =<br>CH= | 5.53 H <sub>1a</sub> and 5.48 H <sub>1b</sub> | d, <i>J</i> = 10 Hz<br>dq, <i>J</i> = 15 Hz, 10 Hz, 5 Hz | 127.0                     | 3.56, 3.70 H <sub>3,ab</sub> ( <i>J</i> <sup>3</sup> )                                                   |
| 2                          |                          | 5.90 H <sub>2a</sub>                          | m                                                            | 127.5                     | 3.60, 3.73 H <sub>3a,b</sub> ( <i>J</i> <sup>3</sup> )<br>5.53 H <sub>1</sub>                         | 2'         |                          | 5.90 H <sub>2a</sub>                          | m                                                        | 127.5                     | 3.56, 3.70 H <sub>3a,b</sub> ( <i>J</i> <sup>3</sup> )<br>5.48 H <sub>1</sub>                            |
| 3                          | CH <sub>2</sub> =        | 3.60 H <sub>3a</sub> and 3.73 H <sub>3b</sub> | dq, <i>J</i> = 10 Hz                                         | 56.2                      | 5.53, 5.48 H <sub>1a,b</sub> ( <i>J</i> <sup>3</sup> )                                                | 3'         | CH <sub>2</sub> =        | 3.56 H <sub>3a</sub> and 3.70 H <sub>3b</sub> | dq, <i>J</i> = 5 Hz, 10 Hz                               | 55.73                     | 5.53, 5.48 H <sub>1a,b</sub> ( <i>J</i> <sup>3</sup> )                                                   |
| 4                          | CH <sub>2</sub> =        |                                               | dq, <i>J</i> = 5 Hz, 10 Hz                                   |                           | 5.90 H <sub>2</sub> ( <i>J</i> <sup>2</sup> ), 3.65, 3.83 H <sub>3,ab</sub> ( <i>J</i> <sup>3</sup> ) | 4'         | CH <sub>2</sub> =        |                                               | dq, <i>J</i> = 5 Hz, 10 Hz                               |                           | 3.78, 3.80 H <sub>4,ab</sub>                                                                             |
|                            | CH=                      | 3.65 H <sub>4a</sub> and 3.83 H <sub>4b</sub> | dq, <i>J</i> = 10 Hz, 5 Hz                                   | 51.5                      | 6.54 H <sub>6a</sub> ( <i>J</i> <sup>2</sup> ), 6.0 H <sub>5a</sub> ( <i>J</i> <sup>2</sup> )         |            | CH=                      | 3.78 H <sub>4a</sub> and 3.80 H <sub>4b</sub> | dq, <i>J</i> = 10 Hz, 5 Hz, 18 Hz                        | 51.3                      | 6.73 H <sub>6a</sub> ( <i>J</i> <sup>2</sup> )                                                           |
|                            | CH=                      |                                               |                                                              |                           | 3.60, 3.73 H <sub>3a,b</sub> ( <i>J</i> <sup>3</sup> )                                                |            | CH=                      |                                               |                                                          |                           | 5.83 H <sub>7a</sub> ( <i>J</i> <sup>2</sup> )<br>3.56, 3.70 H <sub>3,ab</sub> ( <i>J</i> <sup>3</sup> ) |
| 5                          |                          | 5.83 H <sub>5a</sub>                          | dq, <i>J</i> = 5 Hz, <i>J</i> = 15 Hz                        | 121.0                     | 3.65, 3.83 H <sub>3a,b</sub> ( <i>J</i> <sup>3</sup> )                                                | 5'         |                          | 6.00 H <sub>5a</sub>                          | dq, <i>J</i> = 10 Hz                                     | 119.0                     | 3.78, 3.80 H <sub>4,ab</sub> ( <i>J</i> <sup>3</sup> )                                                   |
| 6                          |                          | 6.54 H <sub>6a</sub>                          | d, <i>J</i> = 15 Hz                                          | 137.5                     | 3.65, 3.83 H <sub>3a,b</sub> ( <i>J</i> <sup>3</sup> )                                                | 6'         |                          | 6.73 H <sub>6a</sub>                          | d, <i>J</i> = 10 Hz                                      | 142.0                     | 3.78, 3.80 H <sub>4,ab</sub> ( <i>J</i> <sup>3</sup> )                                                   |
| 7                          | CH <sub>2</sub> =<br>CH= | 3.43 H <sub>7a,b</sub>                        | d, <i>J</i> = 5 Hz                                           | 43.0                      | 5.20, 5.25 H <sub>9a,b</sub> ( <i>J</i> <sup>3</sup> )                                                | 7'         | CH <sub>2</sub> =<br>CH= | 3.45 H <sub>7,ab</sub>                        | d, <i>J</i> = 5 Hz                                       | 44.0                      | 5.20, 5.25 H <sub>9,ab</sub> ( <i>J</i> <sup>3</sup> )                                                   |
| 8                          |                          | 5.95 H <sub>8a</sub>                          | m                                                            | 136.1                     | 3.43 H <sub>7a,b</sub> ( <i>J</i> <sup>3</sup> )                                                      | 8'         |                          | 5.95 H <sub>8a</sub>                          | m                                                        | 136.0                     | 3.45 H <sub>7,ab</sub> ( <i>J</i> <sup>3</sup> )                                                         |
| 9                          | CH <sub>2</sub> =        | 5.20 H <sub>9a,b</sub>                        | d, <i>J</i> = 5 Hz                                           | 122.1                     | 3.43 H <sub>7,ab</sub> ( <i>J</i> <sup>3</sup> )                                                      | 9'         | CH <sub>2</sub> =        | 5.25 H <sub>9,ab</sub>                        | d, <i>J</i> = 5 Hz                                       | 122.0                     | 3.45 H <sub>7,ab</sub> ( <i>J</i> <sup>3</sup> )                                                         |

<sup>a</sup>NMR performed using a Bruker Daltonics maxis 3G instrument, 500 MHz.<sup>b</sup>m, multiplet; dq, doublet of quartets; d, doublet.

Garlic bulbs (*A. sativum* L.; Liliaceae) of the purple-striped variety were purchased in Campos Altos, MG, Brazil from the Fazenda Tri "S" Farm, situated at an altitude of 1.132 m, in November 2017. Samples of the bulbs were cultivated, and the whole plant was subsequently provided to experts at the Botanical Institute of São Paulo, who confirmed its identity. A voucher specimen, no. 502077, was deposited at the Maria Eneyda P. Kaufmann Fidalgo (SP) Herbarium, São Paulo, Brazil.

**Crude garlic extract.** Damage- and fungi-free fresh bulbs (200 g) of *A. sativum* L. were carefully peeled, washed under running water, and placed in a miniprocessor (Cadence, Balneário Piçarras, SC, Brazil) for approximately 1 min. The processed material was filtered through a no. 20 mesh sieve. The liquid thus obtained was freeze-dried (Thermo Super Modulo Pirani 501), stored in a freezer ( $-20^{\circ}\text{C}$ ), and labeled as crude garlic extract (CGE).

**Solid phase extraction.** CGE (2 g) was incubated in 5 ml acetic acid (2M) with magnetic stirring in a refrigerated system (ice bath) for 30 min and then centrifuged at  $14,680 \times g$  for 5 min; the supernatant (4.0 ml) was injected into Sep-Pak  $C_{18}$  cartridges (Waters Corp., Milford, MA, USA) equilibrated in 0.05% trifluoroacetic acid (TFA). The sample was eluted in acetonitrile (ACN; concentration, 80%), then concentrated and labeled as Sep-Pak 80% (SP80) (47).

**Bio-guided fractionation by RP-UFLC-UV.** The bio-guided fractionation of SP80 was performed using a reverse-phase ultrafast liquid chromatography with UV (RP-UFLC-UV) system on a Prominence chromatograph (Shimadzu Corp., Kyoto, Japan) with a preparative reverse-phase (RP) column Shim-pack PREP-ODS (250 mm  $\times$  50 mm inside diameter [i.d.], 15  $\mu\text{m}$ ). The SP80 was reconstituted in 6 ml acidified water (TFA 0.05%) and divided into three runs. Purification of the sample was carried out using a gradient of 0% to 80% ACN at a flow rate of 2.0 ml/min for 120 min. UV absorbance of the effluent was monitored at 225 nm. All eluted peaks were manually collected, vacuum-dried to completely remove the solvent, reconstituted in ultrapure water (250  $\mu\text{l}$ ), and stirred by vortexing and ultrasound for complete dissolution before being used in the antimicrobial activity assays (47).

**LC-DAD-MS/MS/ESI<sup>+</sup> and NMR.** Mass spectrometry analysis was performed on a Shimadzu model CBM-20A chromatograph, equipped with Shimadzu LC-20AD pumps, a Shimadzu SPD-20A detector, Shimadzu CTO-20A oven, Shimadzu SIL 20AC autoinjector, and Bruker Daltonics maXis 3G mass spectrometer (Bruker Corp., Billerica, MA, USA), with a 4500-V capillary, 2-bar nebulizers, at  $200^{\circ}\text{C}$ , and a gas flow of 8 liter/ $\text{min}^{-1}$  operating in MS and MS/MS data acquisition mode with the electron spray source in positive mode (ESI<sup>+</sup>). For the liquid chromatography, a reverse-phase  $C_{18}$  (Phenomenex Luna) plus column (250 mm  $\times$  4.6 mm  $\times$  5  $\mu\text{m}$ ) was used, with a flow rate of 1.0 ml. $\text{min}^{-1}$ . The elution system used a gradient system which consisted of (i) acidified water (TFA 0.05%) and (ii) acetonitrile (ACN) for 10 to 70 min, reaching 80% ACN (48).

Nuclear magnetic resonance (NMR) was performed using a Bruker III 500-MHz spectrometer in deuterated water ( $\text{D}_2\text{O}$ ), and the results were analyzed by unidimensional (1D)  $^1\text{H}$  NMR and two-dimensional (2D) heteronuclear single quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC) (48).

**Culture of *Streptococcus agalactiae*.** *S. agalactiae* culture (1 CFU) was grown in 4 ml of tryptic soy broth (TSB) for 18 h at  $37^{\circ}\text{C}$  overnight. After the medium reached the desired turbidity, 500  $\mu\text{l}$  was transferred into 10 ml TSB and maintained until the exponential growth phase. Bacterial suspensions at  $10^4$  CFU/ml were prepared by adjusting the optical density to  $\text{OD}_{595} = 0.250 \pm 0.005$  (49).

**Liquid growth inhibition test.** The antimicrobial assay was performed against *S. agalactiae* ATCC 12386 using a TSB medium. Antimicrobial activity was determined using microliter broth dilution assays in 96-well sterile plates. The dried fractions were dissolved in 250  $\mu\text{l}$  ultrapure water; then, 20  $\mu\text{l}$  was aliquoted into each well with 80  $\mu\text{l}$  microbial dilution at a final volume of 100  $\mu\text{l}$ . Sterile water and TSB were used as the negative control, and penicillin G (Inlab Confiança, São Paulo, Brazil), the antibiotic of choice in the treatment of *S. agalactiae* infections, was used as the positive control. After 24 h of incubation at  $35 \pm 2^{\circ}\text{C}$ , the growth inhibition was determined by measuring absorbance at 595 nm using a Victor3 1420 instrument (Perkin Elmer).

Five strains of clinical isolates of *S. agalactiae*, kindly supplied by the Salomão Zoppi Clinical Laboratory (São Paulo, Brazil) from its library, were used in the preliminary screening of the action of the fractions with antimicrobial activity (49).

**MIC.** To determine the MIC, the samples, dissolved in ultrapure water, were used to perform serial dilutions in 96-well sterile plates at a final volume of 100  $\mu\text{l}$  against *S. agalactiae* ATCC 12386. For this, 20  $\mu\text{l}$  of the fraction was applied to each well at a serial dilution of 2-fold microtiter broth dilution and added to 80  $\mu\text{l}$  of microbial dilution. The MIC was considered to be the lowest concentration of each sample after 24 h of incubation at  $35 \pm 2^{\circ}\text{C}$  that completely inhibited growth of the microorganism. The tests were performed in triplicate, and the average of three readings was considered (49).

**Bacterial growth curve kinetics.** To evaluate the kinetics of the antimicrobial effects of fractions 18, 20, and 42, the growth curves of *S. agalactiae* ATCC 12386 were determined. Bacteria were plated in 96-well plates containing TSB medium at  $1 \times 10^5$  CFU/well, and 20  $\mu\text{l}$  of the fraction was then applied to each well at concentrations of  $3 \times \text{MIC}$  with 80  $\mu\text{l}$  microbial dilution at a final volume of 100  $\mu\text{l}$ , respectively. The OD value at 595 nm was determined every 1 h (up to 18 h) using a Victor3 1420 instrument (Perkin Elmer). The TSB medium was used as the negative control and penicillin G as the positive control. The experiment was performed in triplicate. After an 18-h incubation, 50  $\mu\text{l}$  of each fraction was plated onto blood agar plates to count the CFU (50).

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K.A.D.M.T. was mainly responsible for the development of the experiments and the writing of the manuscript; S.M.R.R.L. guided the work and contributed to the revision of the final version of the text; L.M.B.T. was responsible for data analysis and interpretation with respect to the LC-DAD-MS/MS/ESI<sup>+</sup> and NMR; M.T.G. was responsible for statistical analysis and the writing of the manuscript; P.I.D.S.J. was responsible for the conception and design of the study. All authors gave their approval for the final version of the text to be submitted.

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We declare no conflicts of interest.

## REFERENCES

- Gizachew M, Tiruneh M, Moges F, Tessema B. 2019. *Streptococcus agalactiae* maternal colonization, antibiotic resistance and serotype profiles in Africa: a meta-analysis. *Ann Clin Microbiol Antimicrob* 18:14. <https://doi.org/10.1186/s12941-019-0313-1>.
- Puopolo KM, Lynfield R, Cummings JJ, Committee on Fetus and Newborn, Committee on Infectious Diseases. 2019. Management of infants at risk for group B streptococcal disease. *Pediatrics* 144:e20191881. <https://doi.org/10.1542/peds.2019-1881>.
- Furfaro LL, Chang BJ, Payne MS. 2018. Perinatal *Streptococcus agalactiae* epidemiology and surveillance targets. *Clin Microbiol Rev* 31:e00049-18. <https://doi.org/10.1128/CMR.00049-18>.
- American College of Obstetricians and Gynecologists (ACOG) Committee on Obstetric Practice. 1996. Prevention of early-onset group B streptococcal disease in newborns. *Int J Gynaecol Obstet* 54:197–205. [https://doi.org/10.1016/S0020-7292\(96\)90083-1](https://doi.org/10.1016/S0020-7292(96)90083-1).
- Centers for Disease Control and Prevention (CDC). 1996. Prevention of perinatal group B streptococcal disease: a public health perspective. *MMWR Recomm Rep* 45:1–24.
- American Academy of Pediatrics (AAP) Committee on Infectious Diseases, Committee on Fetus and Newborn. 1997. Revised guidelines for prevention of early-onset group B streptococcal (GBS) infection. *Pediatrics* 99: 489–496. <https://doi.org/10.1542/peds.99.3.489>.
- Centers for Disease Control and Prevention (CDC). 2010. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC. *MMWR Recomm Rep* 59:1–32.
- Cohain JS. 2009. Long-term symptomatic group B streptococcal vulvovaginitis: eight cases resolved with freshly cut garlic. *Eur J Obstet Gynecol Reprod Biol* 146:110–111. <https://doi.org/10.1016/j.ejogrb.2009.05.028>.
- Cohain JS. 2010. Case series: symptomatic group B streptococcus vaginitis treated with fresh garlic. *Integr Med* 9:40–43.
- Yoo M, Lee S, Lee S, Seog H, Shin D. 2010. Validation of high-performance liquid chromatography methods for determination of bioactive sulfur compounds in garlic bulbs. *Food Sci Biotechnol* 19:1619–1626. <https://doi.org/10.1007/s10068-010-0229-1>.
- Capasso A. 2013. Antioxidant action and therapeutic efficacy of *Allium sativum* L. *Molecules* 18:690–700. <https://doi.org/10.3390/molecules18010690>.
- Nicastro HL, Ross SA, Milner JA. 2015. Garlic and onions: their cancer prevention properties. *Cancer Prev Res (Phila)* 8:181–189. <https://doi.org/10.1158/1940-6207.CAPR-14-0172>.
- Lee DY, Li H, Lim HJ, Lee HJ, Jeon R, Ryu JH. 2012. Anti-inflammatory activity of sulfur-containing compounds from garlic. *J Med Food* 15:992–999. <https://doi.org/10.1089/jmf.2012.2275>.
- Percival SS. 2016. Aged garlic extract modifies human immunity. *J Nutr* 146:433S–436S. <https://doi.org/10.3945/jn.115.210427>.
- Mehrbod P, Amini E, Kheir MT, Pasteur Institute of IRAN (Influenza Unit). 2009. Antiviral activity of garlic extract on influenza virus. *Iran J Virol* 3: 19–23. <https://doi.org/10.21859/iv.3.1.19>.
- Lanzotti V, Scala F, Bonanomi G. 2014. Compounds from *Allium* species with cytotoxic and antimicrobial activity. *Phytochem Rev* 13:769–791. <https://doi.org/10.1007/s11101-014-9366-0>.
- Serrano HDA, Mariezcurrena-Berasain MA, Del Carmen Gutiérrez Castillo A, Carranza BV, Pliego AB, Rojas MT, Anele UY, Salem AZM, Rivas-Caceres RR. 2020. Antimicrobial resistance of three common molecularly identified pathogenic bacteria to *Allium* aqueous extracts. *Microb Pathog* 142: 104028. <https://doi.org/10.1016/j.micpath.2020.104028>.
- Castro C, Lorenzo AG, González A, Cruzado M. 2010. Garlic components inhibit angiotensin II-induced cell-cycle progression and migration: involvement of cell-cycle inhibitor p27 (Kip1) and mitogen-activated protein kinase. *Mol Nutr Food Res* 54:781–787. <https://doi.org/10.1002/mnfr.200900108>.
- Shang A, Cao S-Y, Xu X-Y, Gan R-Y, Tang G-Y, Corke H, Mavumengwana V, Li H-B. 2019. Bioactive compounds and biological functions of garlic (*Allium sativum* L.). *Foods* 8:246. <https://doi.org/10.3390/foods8070246>.
- Lee HS, Lim WC, Lee SJ, Lee SH, Lee JH, Cho HY. 2016. Antiobesity effect of garlic extract fermented by *Lactobacillus plantarum* BL2 in diet-induced obese mice. *J Med Food* 19:823–829. <https://doi.org/10.1089/jmf.2016.3674>.
- Quesada I, de Paola M, Torres-Palazzolo C, Camargo A, Ferder L, Manucha W, Castro C. 2020. Effect of garlic's active constituents in inflammation, obesity and cardiovascular disease. *Curr Hypertens Rep* 22:6. <https://doi.org/10.1007/s11906-019-1009-9>.
- Cavallito CJ, Bailey JH. 1944. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *J Am Chem Soc* 66:1950–1951. <https://doi.org/10.1021/ja01239a048>.
- Choo S, Chin VK, Wong EH, Madhavan P, Tay ST, Yong PVC, Chong PP. 2020. Review: antimicrobial properties of allicin used alone or in combination with other medications. *Folia Microbiol (Praha)* 65:451–465. <https://doi.org/10.1007/s12223-020-00786-5>.
- Nakamoto M, Kunimura K, Suzuki JI, Kodera Y. 2020. Antimicrobial properties of hydrophobic compounds in garlic: allicin, vinylidithiin, ajoene and diallyl polysulfides. *Exp Ther Med* 19:1550–1553.
- Kaminska D, Ratajczak M, Szumala-Kąkol A, Długaszewska J, Nowak-Malczewska DM, Gajecka M. 2020. Increasing resistance and changes in distribution of serotypes of *Streptococcus agalactiae* in Poland. *Pathogens* 9:526. <https://doi.org/10.3390/pathogens9070526>.
- National Center for Biotechnology Information (NCBI). 2021. gamma-Glutamyl-S-allylcysteine (compound). PubChem. <https://pubchem.ncbi.nlm.nih.gov/compound/gamma-Glutamyl-S-allylcysteine>. Accessed on 6 April 2021.
- National Center for Biotechnology Information (NCBI). 2021. Ajoene (compound). PubChem. <https://pubchem.ncbi.nlm.nih.gov/compound/Ajoene>. Accessed on 6 April 2021.
- Cutler RR, Odent M, Hajj-Ahmad H, Maharjan S, Bennett NJ, Josling PD, Ball V, Hatton P, Dall'Antonia M. 2009. In vitro activity of an aqueous allicin extract and a novel allicin topical gel formulation against *Lancefield* group B streptococci. *J Antimicrob Chemother* 63:151–154. <https://doi.org/10.1093/jac/dkn457>.
- Phan ADT, Netzel G, Chhim P, Netzel ME, Sultanbawa Y. 2019. Phytochemical characteristics and antimicrobial activity of Australian grown garlic (*Allium sativum* L.) cultivars. *Foods* 8:358. <https://doi.org/10.3390/foods8090358>.
- Zhu Q, Kakino K, Nogami C, Ohnuki K, Shimizu K. 2016. An LC-MS/MS-SRM method for simultaneous quantification of four representative organosulfur compounds in garlic products. *Food Anal Methods* 9:3378–3384. <https://doi.org/10.1007/s12161-016-0535-1>.

31. Koca I, Tasci B. 2016. Garlic as a functional food. *Acta Hort* 1143: 139–145. <https://doi.org/10.17660/ActaHortic.2016.1143.20>.
32. Dwivedi VP, Bhattacharya D, Singh M, Ashima B, Kumar S, Fatima S, Sobia P, Kaer LV, Das G. 2019. Allicin enhances antimicrobial activity of macrophages during *Mycobacterium tuberculosis* infection. *J Ethnopharmacol* 243:111634. <https://doi.org/10.1016/j.jep.2018.12.008>.
33. Metwally DM, Al-Olayan EM, Alanazi M, Alzahrany SB, Semlali A. 2018. Antischistosomal and anti-inflammatory activity of garlic and allicin compared with that of praziquantel in vivo. *BMC Complement Altern Med* 18: 135. <https://doi.org/10.1186/s12906-018-2191-z>.
34. Kaschula CH, Tuveri R, Ngarande E, Dzobo K, Barnett C, Kusza DA, Graham LM, Katz AA, Rafudeen MS, Parker MI, Hunter R, Schäfer G. 2019. The garlic compound ajoene covalently binds vimentin, disrupts the vimentin network and exerts anti-metastatic activity in cancer cells. *BMC Cancer* 19: 248. <https://doi.org/10.1186/s12885-019-5388-8>.
35. Arnault I, Christidès JP, Mandon N, Haffner T, Kahane R, Auger J. 2003. High-performance ion-pair chromatography method for simultaneous analysis of alliin, deoxyalliin, allicin and dipeptide precursors in garlic products using multiple mass spectrometry and UV detection. *J Chromatogr A* 991:69–75. [https://doi.org/10.1016/s0021-9673\(03\)00214-0](https://doi.org/10.1016/s0021-9673(03)00214-0).
36. Amagase H. 2006. Clarifying the real bioactive constituents of garlic. *J Nutr* 136:716S–725S. <https://doi.org/10.1093/jn/136.3.716S>.
37. Yeh YY, Liu L. 2001. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. *J Nutr* 131: 989S–998S. <https://doi.org/10.1093/jn/131.3.989S>.
38. Wang Y, Sun Z, Chen S, Jiao Y, Bai C. 2016. ROS-mediated activation of JNK/p38 contributes partially to the pro-apoptotic effect of ajoene on cells of lung adenocarcinoma. *Tumour Biol* 37:3727–3738. <https://doi.org/10.1007/s13277-015-4181-9>.
39. Carrero S, Romero H, Apitz-Castro R. 2009. Efecto inhibitorio in vitro de ajoeno sobre aislamientos de *Candida* recuperados de secreciones vaginales. *Rev Iberoam Micol* 26:189–193. (In Spanish.) <https://doi.org/10.1016/j.riam.2009.02.002>.
40. Torres J, Romero H. 2012. In vitro antifungal activity of ajoene on five clinical isolates of *Histoplasma capsulatum* var. *capsulatum*. *Rev Iberoam Micol* 29:24–28. <https://doi.org/10.1016/j.riam.2011.04.001>.
41. Choi JA, Cho SN, Lim YJ, Lee J, Go D, Kim SH, Song CH. 2018. Enhancement of the antimycobacterial activity of macrophages by ajoene. *Innate Immun* 24:79–88. <https://doi.org/10.1177/1753425917747975>.
42. Apitz-Castro R, Badimon JJ, Badimon L. 1992. Effect of ajoene, the major antiplatelet compound from garlic, on platelet thrombus formation. *Throm Res* 68:145–155. [https://doi.org/10.1016/0049-3848\(92\)90030-E](https://doi.org/10.1016/0049-3848(92)90030-E).
43. Kaschula CH, Hunter R, Parker MI. 2010. Garlic-derived anticancer agents: structure and biological activity of ajoene. *Biofactors* 36:78–85. <https://doi.org/10.1002/biof.76>.
44. Hossain MA, Biva I, Kidd SE, Whittle JD, Griesser HJ, Coad BR. 2019. Antifungal activity in compounds from the Australian desert plant *Eremophila alternifolia* with potency against *Cryptococcus* spp. *Antibiotics* (Basel) 8: 34. <https://doi.org/10.3390/antibiotics8020034>.
45. López Y, Parra E, Cepas V, Sanfeliú I, Juncosa T, Andreu A, Xercavins M, Pérez J, Sanz S, Vergara A, Bosch J, Soto SM. 2018. Serotype, virulence profile, antimicrobial resistance and macrolide-resistance determinants in *Streptococcus agalactiae* isolates in pregnant women and neonates in Catalonia, Spain. *Enferm Infecc Microbiol Clin (Engl Ed)* 36:472–477. <https://doi.org/10.1016/j.eimc.2017.08.006>.
46. Velázquez LM, Bobadilla F, Novosak M, Cortese I, Laczkeski M. 2017. Vigilancia de la sensibilidad a penicilina en *Streptococcus agalactiae* recuperados de embarazadas entre las 35–37 semanas de gestación. *Rev Fac Cien Med Univ Nac Cordoba* 74:320–324. (In Spanish.) <https://doi.org/10.31053/1853.0605.v74.n4.16395>.
47. Diniz LCL, Miranda A, da Silva PI, Jr. 2018. Human antimicrobial peptide isolated from *Triatoma infestans* haemolymph, *Trypanosoma cruzi*-transmitting vector. *Front Cell Infect Microbiol* 8:354. <https://doi.org/10.3389/fcimb.2018.00354>.
48. Universidade de São Paulo. Instituto de Química. Central Analítica. Centro Analítico de Instrumentação. 2019. Espectrometria de massas. Ressonância magnética nuclear. <http://ca.iq.usp.br/novo/>. Accessed 5 April 2021. (In Portuguese.)
49. Clinical and Laboratory Standards Institute (CLSI). 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 9th ed. M07-A9. CLSI, Wayne, PA.
50. Niu H, Zhang H, Wu F, Xiong B, Tong J, Jiang L. 2021. Proteomics study on the protective mechanism of soybean isoflavone against inflammation injury of bovine mammary epithelial cells induced by *Streptococcus agalactiae*. *Cell Stress Chaperones* 26:91–101. <https://doi.org/10.1007/s12192-020-01158-1>.